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Effect of vitamin E and carvedilol in ameliorating gentamicin-induced nephrotoxicity in rabbit

*Rana M.Khalaf

ABSTRACT:

Background: Gentamicin is an antibiotic effective against gram negative infections, whose clinical use is limited by its nephrotoxicity. In fact, the incidence of renal dysfunction during the course of clinical treatment with gentamicin antibiotics is approximately 10%. Direct proximal tubular necrosis and oxidative stress were the main pathogenic factors

.

Aim: to investigate the effect of vitamin E and carvedilol pretreatment in ameliorating gentamicin- induced nephrotoxicity in rabbit.

Materials and methods: eighteen local domestic male rabbits were used; they were separated to three groups, one of them served as a control group. All animals were injected with gentamicin 80 mg/Kg intramuscularly two hours after administration of the tested agent which was distilled water in the control group, vitamin E 200mg/Kg in the second group and carvedilol 3 mg/Kg in the third one for 6 successive days. Renal function was assessed at day 7 by estimating blood urea nitrogen(BUN), serum creatinin, serum potassium and sodium.

Results: treatment with vitamin E and carvedilol prior to nephrotoxic dose of gentamicin results in significant reduction in the levels of BUN, serum creatinin and serum potassium with significant elevation in serum sodium level when compared with the control group.

Conclusion: vitamin E, and carvedilol at the tested doses, have significant nephroprotective effect against gentamicin induced nephrotoxicity in rabbit with possible role in preventing such type of renal insult.

Keywords: gentamicin, nephrotoxicity, prevention, vitamin E, carvedilol.

*M.B.Ch.B, M.Sc in pharmacology.Department of pharmacology and therapeutics- College of medicine-Thi-Qar University

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INTRODUCTION:

Gentamicin is an aminoglycoside antibiotic widely used for the treatment of Gram-negative infections and is a well-known cause of renal failure, which occurs in 10-20% of patients receiving this drug [(1)]. Gentamicin-induced nephrotoxicity is characterized by tubular necrosis mainly involving proximal tubules [(2, 3)], with a marked decrease in glomerular filtration rate, ultrafiltration coefficient and glomerular plasma flow.[(4)]

Pathophysiology:

Nephrotoxicity occurs as a result of proximal tubular nicrosis and glumerular dysfunction. It is because gentamicin ability to generate reactive oxygen species(ROS), so It has been concluded that oxidative stress is involved in gentamicin-induced renal damage. [(3)] Aminoglycosides, which are strongly cationic drugs, bind to the negatively charged acidic phosphoinositide components of the brush border membrane of the proximal tubule. At this site, they reach the cationic drug receptor megalin (gp330) located deep at the base of the brush border villi. This receptor-drug complex is rapidly internalised by pinocytosis and taken up by the lysosomes, where a process of lysosomal phospholipidosis occurs, resulting in the formation of typical morphologic myeloid bodies. The development of phospholipidosis is accompanied by focal necroses, tubular regeneration, and interstitial proliferation, even at low, therapeutic doses of these agents. These changes are related to the nephrotoxic potential of the aminoglycosides. [(9, 10. 11)]

Clinical features:

The most common clinical presentstion is non-oliguric acute renal failure which recover slowly over several weeks, Proximal tubular dysfunction, Hypomagnesaemia,Hypocalcaemia, Hypokalaemia

Treatment and prevention:

Specific and effective strategies are necessary to treat or prevent nephrotoxicity induced by gentamicin.The initial therapy of aminoglycoside-induced acute tubular necrosis is basically supportive,i.e discontinuation of the aminoglycoside and other nephrotoxic agents,maintaining fluid and electrolyte balance, and controlling sepsis. Some of the patients may need dialysis[(12)]To prevent aminoglycoside-induced nephrotoxicity in clinical practice, these drugs should be used at the lowest effective dose for shortest course of therapy, together with renal function monitoring, adequate hydration and avoidance of concomitant nephrotoxic drugs should be kept in mind.[(12,13,14,15)] Gentamicin-induced nephrotoxicity has been only prevented in clinical practice by the use of antioxidants such as n-acetylcysteine [(16)].

MATERIALS AND METHODOLOGY:

Eighteen local domestic male rabbits weighing 750-1000 grams were used in this study. They were purchased from local market. They were fed standard oxoid pellets and were given water *Ad libitum*. The animals were separated into three groups (each group contained six animals), each group was kept in a separate cage.

Animal grouping and treatment schedules:

The groups were treated by giving the tested agents two hours prior to gentamicin injection. Gentamicin was

given in a dose of 80mg/Kg intramuscularly for 6 days [17]. The effect of the tested agents on gentamicin induced nephrotoxicity was investigated by biochemical monitoring of renal function. The treatment schedules were as follows:

Group One: the control group received 5 ml of distilled water orally 2 hours before injection of gentamicin (pallorin-Hemofarm/80mg/2 ml ampules)

Group Two: was given vitamin E (misavit E-mission pharmaceutical limited/400mg tablet) 200 mg/Kg in a single daily dose orally given two hours prior to each gentamicin injection.

Group Three: given carvedilol (carvipress-microLABs limited/6.25mg tablet)
3 mg/Kg as a single dose orally two hour before each gentamicin injection.

Blood was aspirated from the marginal ear vein for two occasions, day 0 to measure the parameters of renal function before treatment, namely serum creatinin, blood urea nitrogen (BUN), serum K^+ and Na^+, and day 7 to demonstrate any effect of the tested agents on renal function.

Statistical analysis: data are expressed as mean±standered deviation, statistical significance among groups was determined by unpaired student's T test using $p<0.05$ as a criterion for significance.

RESULTS:

The results of this study revealed significant elevation in the levels of blood urea nitrogen(BUN) (7.1 ±0.3 versus 4 ± 0.7 mmol/L), serum creatinine(160 ± 2 versus 65 ± 8.9 µmol/L) and serum K^+(5.1 ± 0.4 versus 3.3 ± 0.8 mmol/L) with significant reduction of serum Na^+levels(135± 2 versus 160 ± 4 mmol/L) ($p<0.05$) in control group as compared to the levels measured before treatment (see table1,2,3,4,5).

The results of group 2 showed significant decrease in the levels of BUN(4.5 ± 0.2, versus 7.1 ± 0.3 mmol/L), serum.creatinine(95 ± 3.1 versus 160 ± 2 µmol/L) and serum. K^+ (3.5 ± 0.3 versus 5.1 ± 0.4 mmol/L) ($p<0.05$) and significant increase in serum.Na^+levels(155± 2.5versus 135 ± 2 mmol/L) ($p<0.05$) in comparison to the control group after 6 days of vitamin E administration together with gentamicin injection.

The results of group 3 showed significant reduction of BUN(5.1 ± 0.2versus 7.1 ± 0.3 mmol/L), serum.creatinine(102± 2.6 versus 160 ± 2 µmol/L) and serum. K^+ (3.8 ± 0.2versus 5.1 ± 0.4 mmol/L) ($p<0.05$) and significant increase in serum.Na^+levels(157 ± 3 versus 135 ± 2 mmol/L) ($p<0.05$) in comparison to the control group after 6 days of treatment. (See table2,3,4,5 and figure 1,2,3,4).

DISCUSSION :

Aminoglycoside antibiotics play an integral role in antimicrobial chemotherapy. Unfortunately, these drugs are known to cause nephrotoxicity in man and experimental animals. Gentamicin as one of the aminoglycoside antibiotics exerts its toxic effect directly on the proximal tubule causing acute tubular narcosis. This toxic effect was found to be mediated by generating reactive oxygen species (ROS) [18]. For the control group, gentamicin injection in a dose of 80 mg/kg for 6 successive days resulted in significant impairment in renal function manifested biochemically as 177% increase in BUN, 246% increase in

serum creatinin, 154% increase in serum K^+ and 84%reduction in serum Na^+ levels, these changes demonstrated the nephrotoxic potential of gentamicin.

As generation of reactive oxygen species was diagnosed as the main pathogenic factor in this type of nephrotoxicity, Many antioxidants have been investigated for their nephroprotective effect in gentamicin nephrotoxicity and other models of nephrotoxicity where oxidative stress found to be the main event, of those antioxidants, vitamins namely vitamin C, A and E were extensively investigated and found to be effective in protecting renal tissue against a variety of insults [19, 20]. vitamine E or α-tocopherol is the most important lipid-soluble antioxidant vitamin, that it protects membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction.[21,22] This removes the free radical intermediates and prevents the propagation reaction from continuing. This is in line with findings showing that α-tocopherol, efficiently protects glutathione peroxidase 4 (GPX4)-deficient cells from cell death [23]. GPx4 is the only known enzyme that efficiently reduces lipid-hydroperoxides within biological membranes. Upon gentamicin treatment GPX4 and manganese superoxide dismutase enzyme (Mn-SOD) are reduced together with increased lipid peroxidation [24] . In group 2, vitamin E showed significant nephroprotective effect against gentamicin induced nephrotoxicity at the tested dose and schedule by preventing the significant increase in BUN, serum creatinin and K^+ and preventing the significant reduction in serum Na^+ levels ($P<0.05$), such effect can be attributed to the above mentioned antioxidant action. These results agreed with those of [25] who found such effect of vitamin E in cyclosporine A induced nephrotoxicity in rat, and [26] Who concluded that vitamin E exerted a nephroprotective effect in diabetic nephropathy.Carvedilol is a novel cardiovascular drug of proven efficacy in the treatment of hypertension, angina, and heart failure. It has an alpha and beta adrenoceptor blocking activity; it is a potent antioxidant and is unique among β-blockers in this respect. Although carvedilol blocks beta adrenoceptors, unlike other beta blockers, Carvedilol had no effect on renal hemodynamic parameters; glomerular filtration rate, renal blood flow, and filtration fraction [27].In group 3,carvedilol elicited significant nephroprotective effect at the tested dose and schedule, such protective action demonstrated by significant reduction of BUN, serum creatinin and serum potassium with significant elevation in serum sodium ($P<0.05$) in comparison with the control group, this protective action can be explained by its antioxidant potential. This result agreed with those of [28] who concluded that carvedilol possesses a nephroprotective action against cyclosporine induced nephrotoxicity, and [29] who found that carvedilol is a potential drug for the adjuvant nephroprotective therapy during cisplatin chemotherapy.

CONCLUSION:

In conclusion vitamin E, and carvedilol at the tested doses, have significant nephroprotective effect against gentamicin induced nephrotoxicity in rabbit highlighting possible role in preventing this type of nephrotoxicity.

RECOMMENDATIONS:

It is recommended that further

investigation of vitamin E and carvedilol, in gentamicin-induced nephrotoxicity in human, is required as a preliminary step in their role for preventing this type of important gentamicin toxicity in clinical practice, especially in high risk patients.

Table (1): the mean BUN, serum creatinine, K^+ and Na^+ levels of the tested animals measured before gentamicin injection

Analyte	*Mean level*
BUN	4 ± 0.7 mmol/L
S. creatinine	65 ± 8.9 µmol/L
S. K^+	3.3 ± 0.8 mmol/L
S. Na^+	160 ± 4 mmol/L

Table (2): mean BUN levels of the studied groups measured at day 7 of treatment

Group	*Agent*	*Dose*	*BUN (mmol/L)*
1(control)	Distilled water	3 ml	7.1 ± 0.3
2	Vitamin E	200 mg/Kg	4.5 ± 0.2
3	carvedilol	3mg/Kg	5.1 ± 0.2

Table (3): mean serum Creatinin levels of the studied groups measured at day 7 of treatment

Group	*Agent*	*Dose*	*S.Cr.(µmol/L)*
1(control)	Distilled water	3 ml	160 ± 2
2	Vitamin E	200 mg/Kg	95 ± 3.1
3	carvedilol	3 mg/Kg	102± 2.6

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Table (4): mean serum K+ levels of the studied groups measured at day 7 of treatment

Group	Agent	Dose	S. K+ (mmol/L)
1(control)	Distilled water	3 ml	5.1 ± 0.4
2	Vitamin E	200 mg/Kg	3.5 ± 0.3
3	carvedilol	3 mg/Kg	3.8 ± 0.2

Table (5): mean serum Na+ levels of the studied groups measured at day 7 of treatment

Group	*Agent*	*Dose*	*S. Na (mmol/L)*
1(control)	Distilled water	3 ml	135 ± 2
2	Vitamin E	200 mg/Kg	155± 2.5
3	carvedilol	3 mg/Kg	157 ± 3

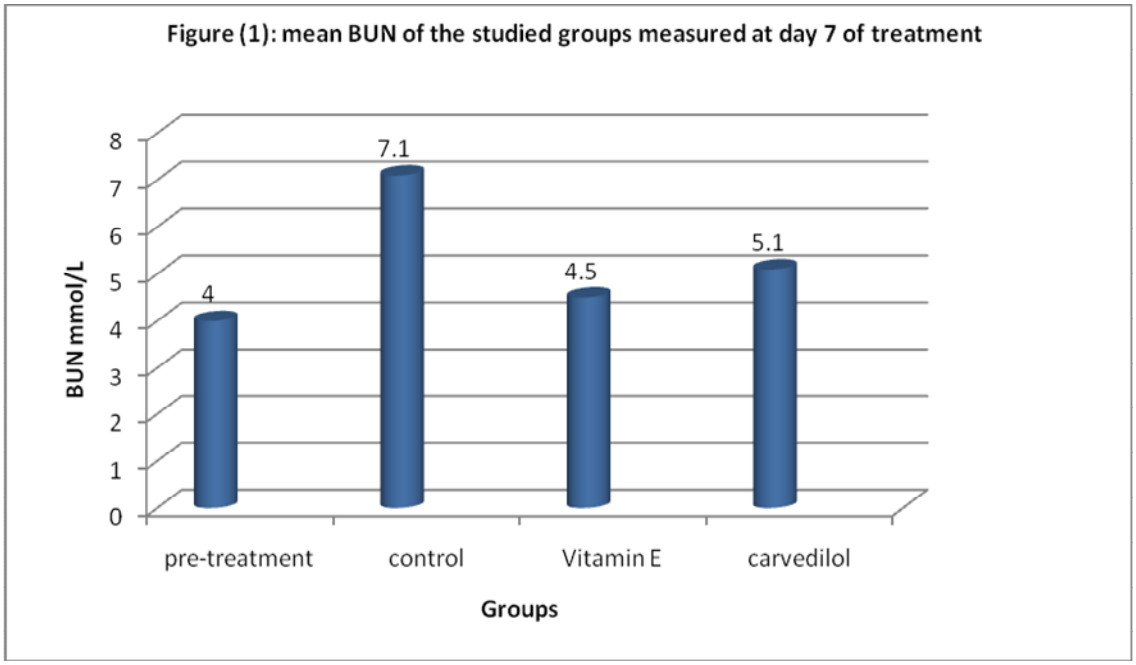


Figure (2): mean serum Creatinin levels of the studied groups measured at day 7 of treatment

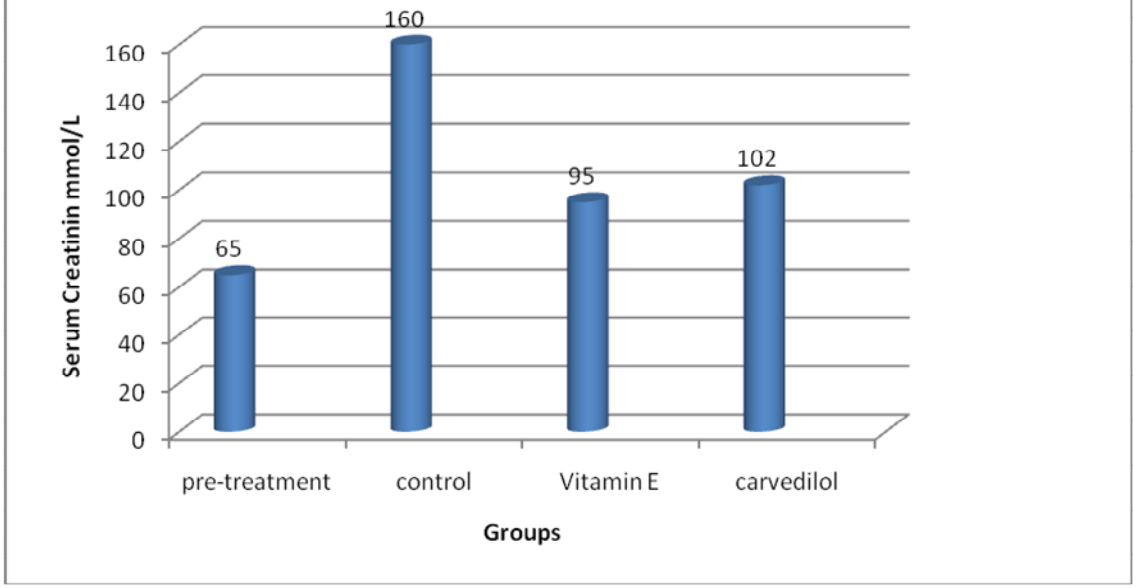
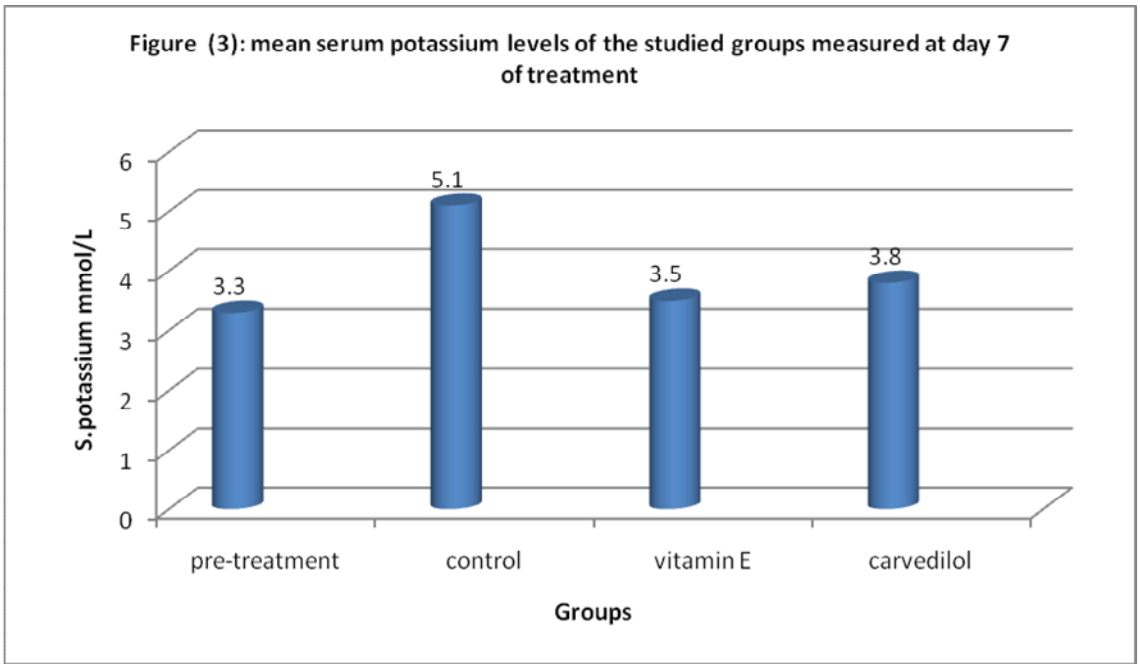
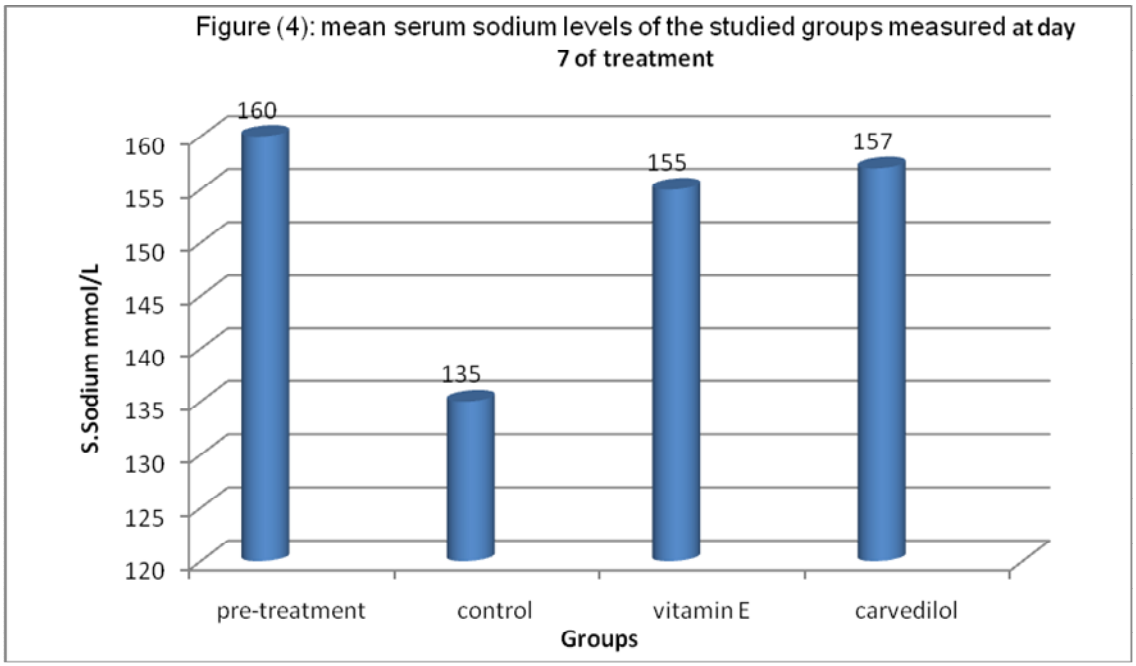


Figure (3): mean serum potassium levels of the studied groups measured at day 7 of treatment





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دراسة تأثير فيتامين أي وعقار الكارفيدايلول في التقليل من شدة سمية الكلية الناجمة عن الجنتامايسين في الارنب

***د. رنا محسن خلف**

ملخص البحث:

تمهيد: يعتبر الجنتامايسين من المضادات الحيوية المهمة في علاج الانتانات الناجمة عن البكتيريا السالبة لصبغة غرام، إلا إن استخدام هذا المضاد الحيوي مقيد بما يسببه من تأثير سمي خاصة تأثيره السام للكلية و الذي يحدث بمعدل ١٠-٢٠% خلال فترة تعاطي الجنتامايسين.
يسبب الجنتامايسين نخر مباشر للنبيب الكلوي الداني وذلك من خلال تحريره جذور الأوكسجين الحرة ،لذا يعتبر الاجهاد التأكسدي الذي يسببه الجنتامايسين السبب الرئيس في سمية الكلية

الهدف: تم اجراء هذه الدراسة من اجل تحري تأثير كل من فيتامين أي وعقار الكارفيدايلول في التقليل من شدة سمية الكلية الناجمة عن الجنتامايسين.

الطرق: اختيرالارنب كنموذج تجريبي لسمية الجنتامايسين الكلوية، تم استخدام ثمانية عشر ارنب محلي ذكر تراوحت معدلات اوزانهم بين ٧٥٠ غم و ١٠٠٠ غم. قسمت الارانب الى ثلاث مجاميع كانت احداها مجموعة سيطرة.حقنت الحيوانات عضليا بالجنتامايسين و بجرعة ٨٠ ملغم\كغم و لمدة ستة ايام متتالية، عولجت الحيوانات بالمواد المراد فحصها قبل ساعتين من الحقن اليومي للجنتامايسين كالاتي: الماء المقطر لمجموعة السيطرة، فيتامين أي ٢٠٠ ملغم\كغم في المجموعة الثانية وعقار الكارفيدايلول ٣ ملغم\كغم في المجموعة الثالثة. قيمت وظيفة الكلية من خلال قياس مستوى اليوريا في الدم ومستوى كل من الكرياتنين و البوتاسيوم و الصوديوم في مصل الدم في اليوم السابع من المعالجة، قورنت النتائج بالقيم السوية و قيم مجموعة السيطرة لتحري تاثير المواد المختبرة الواقي للكلية.

النتائج:بينت النتائج ان المعالجة اليومية ب فيتامين أي ٢٠٠ ملغم\كغم و الكارفيدايلول ٣ ملغم\كغم فمويا سببت نقصا معتد به في مستويات اليوريا والكرياتنين و البوتاسيوم وارتفاع مستوى الصوديوم في مصل الدم عند مقارنتها مع مجموعة السيطرة.

الاستنتاج: من النتائج المتقدمة تبين ان استخدام فيتامين أي وعقار الكارفيدايلول بالجرع المذكورة له تاثير واقي ضد سمية الكلية الناجمة عن الجنتامايسين الامر الذي قد يكون له دور مهم في منع هذا النوع من السمية الكلوية.

مفتاح الكلمات: الجنتامايسين، سمية الكلية، تقليل، فيتامين أي ، الكارفيدايلول.

- فرع الادوية –كلية الطب-جامعة ذي قار*